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### **III. REMARKS**

#### **Preliminary Remarks**

Reconsideration and allowance of the present application based upon the foregoing amendment and following remarks are respectfully requested. Claims 1-21 are currently pending in this application and remain at issue. This response is timely filed.

In paragraph 1 of the official action, the examiner objected to the specification for lacking a specific reference to a prior application wherein its status had not been updated. The applicants have amended the specification to update the status of U.S. Patent Appl. No. 09/431,099, filed November 1, 1999, which is now U.S. Patent No. 6,410,705.

The applicants have also amended the specification on page 20, line 2 to correct a typographical error with regard to the European Patent Application Number cited in that paragraph. The strain DM368-2 is described in EP Patent Appl. No. 0358940 rather than 0385940. European Patent Application Number 0385940 refers to a different technical field, namely a slurry removal of water. Accordingly, the amendment to the specification on page 20, line 2 adds no new matter and does not relate to patentability of the newly claimed invention.

In paragraphs 6-10, the examiner numerously objected claims 6-14 and provided suggestions for appropriate corrections. Solely to expedite prosecution and without prejudice to the applicants right to seek broader claims in a continuing application, the applicants have canceled claims 6-18 without prejudice. New claims 19-60 have been added and contain language that the applicants believe is more consistent with the examiner's comments and for which the applicants are grateful.

The applicants do not intend by these or any amendments to abandon subject matter of the claims as originally filed or later presented, and reserve the right to pursue such subject matter in continuing applications.

#### **Patentability Remarks**

##### **Rejection Pursuant to 35 U.S.C. §112, Second Paragraph**

In paragraph 13 of the official action, claims 6-14 and 16-18 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

Specifically, the examiner rejected a number of wording and phrases in claims 6-14 and 16-

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18. For example, the examiner alleged the recitation of "the thrE gene" was indefinite because this nomenclature for genes encoding proteins of identical function in other organisms may not be accurate. The examiner speculated genes encoding proteins of identical function in two different organisms may use different organisms. The examiner also alleged the term "overexpressed" is a relative term and the specification fails to define this term. Finally, the examiner alleged the phrase "remaining genes of the metabolic pathway for threonine formation are amplified individually or jointly" is indefinite because the specification fails to describe which genes in the metabolic pathway of threonine formation are to be used.

Solely to expedite prosecution and without prejudice to the applicants' right to seek broader claims in a continuing application, the applicants have canceled claims 6-14, and 16-18. New claim 19 is directed to a process for the preparation of L-threonine comprising fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* in which the *Corynebacterium glutamicum* thrE gene encoding a threonine export protein is overexpressed by increasing the copy number of said gene, and isolating said L-threonine produced by said *Corynebacterium* or *Brevibacterium*. New claims 20-25 are directed to the process of claim 19 wherein either the *Corynebacterium glutamicum* pyc gene encoding pyruvate carboxylase, hom gene encoding for homoserine dehydrogenase, hom<sup>dr</sup> allele encoding a feedback-resistant homoserine dehydrogenase, or the mgo gene encoding malate:quinone oxidoreductase are also overexpressed by increasing the copy number in *Corynebacterium* or *Brevibacterium* bacteria and can be of the species *Corynebacterium glutamicum* or *Brevibacterium flavum*. Support for claims 19-25 can be found throughout the specification, for example, original claims 6-14, page 12, lines 23 and 24, and page 10, lines 1-16.

New claim 26 is directed to a process for the preparation of L-threonine comprising fermenting L-threonine producing coryneform bacteria in which the *Corynebacterium glutamicum* thrE gene encoding a threonine export carrier protein is overexpressed by increasing the copy number of the thrE gene, and isolating said L-threonine produced by said coryneform bacteria, wherein said coryneform bacteria have been transformed with a plasmid vector comprising the *C. glutamicum* thrE gene encoding said threonine export carrier protein and said plasmid vector is pZ1thrE, which is deposited in *Brevibacterium flavum* under deposit number DSM12840. New claims 27-34 are directed to the process of claim 27 wherein either the *Corynebacterium glutamicum* pyc gene encoding pyruvate carboxylase, hom gene encoding for homoserine dehydrogenase, hom<sup>dr</sup> allele encoding a feedback-resistant homoserine dehydrogenase, the mgo gene encoding malate:quinone oxidoreductase

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are also overexpressed by increasing the copy number in coryneform bacteria are of the genus *Corynebacterium* or *Brevibacterium* and can be of the species *Corynebacterium glutamicum* or *Brevibacterium flavum*. Support for claims 26-34 can be found throughout the specification, for example, original claims 6-14, page 10, lines 1-16, and page 12, lines 19 - 26.

New claim 35 is directed to a process for the fermentative preparation of L-threonine comprising: (a) fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* in which a *thrE* gene encoding a threonine export carrier protein is overexpressed by increasing the copy number of said gene; and, wherein said coryneform bacteria also overexpress by increasing the copy number of one or more of the coryneform genes selected from the group consisting of: a *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase, a *Corynebacterium glutamicum hom* gene encoding for homoserine dehydrogenase, a *Corynebacterium glutamicum hom<sup>dr</sup>* allele encoding a feedback-resistant homoserine dehydrogenase, and a *Corynebacterium glutamicum mqo* gene encoding for malate:quinone oxidoreductase; (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b). Support for new claim 35 can be found throughout the specification.

New claim 36 is directed to a process for the fermentative preparation of L-threonine comprising (a) fermenting L-threonine producing coryneform bacteria in which a *thrE* gene encoding a threonine export carrier protein is overexpressed by increasing the copy number of the gene; and, wherein said coryneform bacteria also overexpress by increasing the copy number of one or more of the coryneform genes selected from the group consisting of: a *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase, a *Corynebacterium glutamicum hom* gene encoding for homoserine dehydrogenase, a *Corynebacterium glutamicum hom<sup>dr</sup>* allele encoding a feedback-resistant homoserine dehydrogenase, and a *Corynebacterium glutamicum mqo* gene encoding for malate:quinone oxidoreductase (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b) wherein said coryneform bacteria have been transformed with a plasmid vector comprising the *C. glutamicum thrE* gene encoding said threonine export carrier protein and said plasmid vector is pZ1*thrE*, which is deposited in *Brevibacterium flavum* under deposit number DSM12840. Support for new claim 36 can be found throughout the specification.

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New claim 40 is directed to a process for the preparation of L-threonine comprising fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* in which the *Corynebacterium glutamicum thrE* gene encoding a threonine export protein is overexpressed by operatively linking said gene to a promoter, and isolating said L-threonine produced by said *Corynebacterium* or *Brevibacterium*. New claims 41-46 are directed to the process of claim 40 wherein either the *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase, *hom* gene encoding for homoserine dehydrogenase, *hom<sup>dr</sup>* allele encoding a feedback-resistant homoserine dehydrogenase, or the *mgo* gene encoding malate:quinone oxidoreductase are also overexpressed by operatively linking said genes to a promoter in *Corynebacterium* or *Brevibacterium* bacteria and can be of the species *Corynebacterium glutamicum* or *Brevibacterium flavum*. Support for claims 40-46 can be found throughout the specification, for example, original claims 6-14, page 3, lines 3-10, page 12, lines 19-26, and page 10, lines 1-16.

New claim 47 is directed to a process for the preparation of L-threonine comprising fermenting L-threonine producing coryneform bacteria in which the *Corynebacterium glutamicum thrE* gene encoding a threonine export carrier protein is overexpressed by operatively linking the *thrE* gene to a promoter, and isolating said L-threonine produced by said coryneform bacteria, wherein said coryneform bacteria have been transformed with a plasmid vector comprising the *C. glutamicum thrE* gene encoding said threonine export carrier protein and said plasmid vector is pZ1thrE, which is deposited in *Brevibacterium flavum* under deposit number DSM12840. New claims 48-55 are directed to the process of claim 47 wherein either the *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase, *hom* gene encoding for homoserine dehydrogenase, *hom<sup>dr</sup>* allele encoding a feedback-resistant homoserine dehydrogenase, the *mgo* gene encoding malate:quinone oxidoreductase are also overexpressed by operatively linking said genes to a promoter in coryneform bacteria, which are of the genus *Corynebacterium* or *Brevibacterium* and can be of the species *Corynebacterium glutamicum* or *Brevibacterium flavum*. Support for claims 26-34 can be found throughout the specification, for example, original claims 6-14, page 3, lines 3-10, page 10, lines 1-16, and page 12, lines 23 and 24.

New claim 56 is directed to a process for the fermentative preparation of L-threonine comprising: (a) fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* in which a *thrE* gene encoding a threonine export carrier protein is overexpressed operatively linking said gene to a promoter; and, wherein said coryneform bacteria also overexpress by

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operatively linking one or more of the coryneform genes selected from the group consisting of: a *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase, a *Corynebacterium glutamicum hom* gene encoding for homoserine dehydrogenase, a *Corynebacterium glutamicum hom<sup>dr</sup>* allele encoding a feedback-resistant homoserine dehydrogenase, and a *Corynebacterium glutamicum mgo* gene encoding for malate:quinone oxidoreductase to a promoter; (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b). Support for new claim 56 can be found throughout the specification, for example page 3, lines 3-10, page 10, lines 1-16, and page 12, lines 23 and 24.

New claim 57 is directed to a process for the fermentative preparation of L-threonine comprising (a) fermenting L-threonine producing coryneform bacteria in which a *thrE* gene encoding a threonine export carrier protein is overexpressed by operatively linking said gene to a promoter; and, wherein said coryneform bacteria also overexpress by operatively linking one or more of the coryneform genes selected from the group consisting of: a *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase, a *Corynebacterium glutamicum hom* gene encoding for homoserine dehydrogenase, a *Corynebacterium glutamicum hom<sup>dr</sup>* allele encoding a feedback-resistant homoserine dehydrogenase, and a *Corynebacterium glutamicum mgo* gene encoding for malate:quinone oxidoreductase to a promoter, (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b) wherein said coryneform bacteria have been transformed with a plasmid vector comprising the *C. glutamicum thrE* gene encoding said threonine export carrier protein and said plasmid vector is pZ1thrE, which is deposited in *Brevibacterium flavum* under deposit number DSM12840. Support for new claim 57 can be found throughout the specification for example, on page 3, lines 3-10, page 10, lines 1-16, and page 12, lines 19-26.

Finally, new claims 37-39 and 58-60 are directed to either the process of claims 19 or 40 wherein said *thrE* gene comprises a polynucleotide encoding a protein the amino acid sequence of SEQ ID NO: 2, or said polynucleotide can comprise nucleotides 398 to 1864 of SEQ ID NO: 1, or said polynucleotide can comprise SEQ ID NOS: 1 and 3. Support for new claims 37-39 and 58-60 can be found throughout the specification, for example, on page 17, lines 14-19.

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The applicant respectfully submits that new claims 19-60 contains language that clarifies the claimed invention and does not harbor the same alleged indefiniteness issues for canceled claims 6-14, and 16-18. Accordingly, in view of the foregoing amendment, the applicants respectfully submit the rejection of claims 6-14, and 16-18 under 35 U.S.C. §112, second paragraph, is moot and should be withdrawn, a rejection of new claims 19-60 would be improper.

**Rejection Pursuant to 35 U.S.C. §112, First Paragraph**

*Written Description*

In paragraph 20 of the official action, the examiner rejected claims 6-14, and 16-18 under 35 U.S.C. §112, first paragraph, for allegedly failing to comply with the written description requirement. Specifically, the examiner alleged that while the specification discloses the structure of the *C. glutamicum thrE* gene and the production of threonine with *B. flavum* transformed with a plasmid containing the *C. glutamicum thrE* gene, the specification does not disclose the structure of other genes encoding threonine export proteins, the structures of other coryneform bacterial genes involved in threonine biosynthesis, the methods for partially switching off the metabolic pathways, methods for increasing intracellular activity, and all the metabolite or antimetabolite resistance mutations in any coryneform bacteria encompassed by the claims.

As discussed above, claims 6-14 and 16-18 have been canceled without prejudice. New claims 19-60 directed to the claimed process as described above using *C. glutamicum thrE* gene in *Corynebacterium* or *Brevibacterium*, which the examiner acknowledged as being fully supported and enabled by the specification (see Official Action, page 8, lines 1-3, and page 9, 18-24). For example, independent claims 19, 26, 33, and 34 are all directed to a process of isolating L-threonine by overexpressing the *C. glutamicum thrE* gene by increasing the copy number. Dependent claims 20-26, and 28-32 are further supported by the teachings of the specification as other *C. glutamicum* genes which can be overexpressed in tandem with the overexpression of the *thrE* gene (see specification at page 10, lines 1-16). For example, other *C. glutamicum* genes, like the *hom<sup>dr</sup>*, are readily described and available in strains like DSM 368-2 (see specification on page 10, lines 7-11, and page 20, lines 1 and 2). Independent claims 40, 47, 56, and 57 are all directed to a process of isolating L-threonine by overexpressing the *C. glutamicum thrE* gene by operatively linking the *thrE* gene to a promoter. Dependent claims 41-46, and 48-54 are further supported by the

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teachings of the specification as other *C. glutamicum* genes are overexpressed in the same manner as the *thrE* gene (see specification on page 3, lines 3-10. Accordingly, in view of the foregoing amendments and remarks, the rejection of claims 6-14, and 16-18 pursuant to 35 U.S.C. §112, first paragraph, for allegedly lacking written description is moot and should be withdrawn, and a rejection of new claims 19-60 would be improper.

#### *Enablement*

In paragraph 21 of the official action, the examiner rejected claims 6-14 and 16-18 pursuant to 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement by the specification. The examiner alleged that while the specification was enabling for a method for producing L-threonine wherein the method comprises fermentation of *C. glutamicum* or *B. flavum* transformed with a plasmid containing the *C. glutamicum thrE* gene, wherein the *thrE* gene is overexpressed by increasing the copy number of said gene, the specification does not reasonably provide enablement for the claimed invention. Specifically, the examiner asserted the specification lacked enablement for a method for producing L-threonine wherein the method comprises fermentation of any coryneform bacteria wherein the bacteria comprises any gene encoding a threonine export carrier protein, and wherein the intracellular activity of other proteins encoded by any gene involved in threonine biosynthesis is increased by any means. The examiner concluded that based on the specification's teachings, it does not enable any person skilled in the art to make and/or use the invention commensurate in scope with these claims.

As stated above, the applicants have canceled claims 6-14, and 16-18 without prejudice. New claims 19-39 are directed to the claimed process as described above which the examiner has acknowledged as being fully supported and enabled by the specification (see Office Action, page 9, lines 18-24). New claims 40-60 are directed to claimed processes which are well within the routine skills of one of ordinary skill in the art. Accordingly, one of skill in the art could routinely perform and described the claimed invention according to the teachings of the specification. In view of the foregoing amendment and remarks, the applicants respectfully submit that the rejection of claims 6-14 and 26-28 under 35 U.S.C. §112, first paragraph, for allegedly lacking enablement has been overcome, and should be withdrawn, and a rejection of new claims 19-60 would be improper.

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**Rejection Pursuant Judicially Created Doctrine of Obviousness-Type Double Patenting**

In paragraph 23 of the official action, the examiner rejected claims 6-8, 10-14, and 16-18 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 6 of U.S. Patent No 6,596,516. Specifically, the examiner alleged claim 6 of U.S. Patent No. 6,596,516 is directed to a fermentation process for the production of L-threonine wherein said bacterial attenuated *glyA* gene and wherein one or more genes selected from the group consisting of (a) gene encoding homoserine dehydrogenase, (b) gene encoding glyceraldehyde 3 phosphate dehydrogenase, (c) gene encoding threonine export protein, (d) gene encoding malate:quinone oxidoreductase, and (e) gene encoding threonine export carrier protein are overexpressed. The applicants respectfully traverse the rejection.

Again, as discussed above, solely to expedite prosecution and without prejudice to the applicants' right to seek broader claims in a continuing application, the applicants have canceled claims 6-8, 10-14, and 16-18 thereby rendering the rejection under the judicially created doctrine of obviousness-type double patenting moot.

The applicants further submit, however, an extension of this rejection to new claims 19-34 would be improper. Specifically, the applicants submit **that nowhere in the specification or the claimed invention** as defined by new claims 19-60 is there a discussion or teaching of combining the attenuated *glyA* gene with one or more overexpressed (via copy number or operatively linking to a promoter) genes selected from the group consisting of *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase, *Corynebacterium glutamicum hom* gene encoding for homoserine dehydrogenase, *Corynebacterium glutamicum hom<sup>dr</sup>* allele encoding a feedback-resistant homoserine dehydrogenase, the *Corynebacterium glutamicum mqo* gene encoding malate:quinone oxidoreductase. Rather, the applicants' invention is directed to a fermentative process in *Corynebacterium* or *Brevibacterium* wherein the *C. glutamicum thrE* gene is overexpressed, and may be combined with the overexpression of one of the following *C. glutamicum* genes: *pyc*, *hom*, *hom<sup>dr</sup>* allele, and *mqo*.

The specification does not even mention attenuating or for that matter using the *glyA* gene to which U.S. Patent No 6,596,516 is directed to fermentation production of L-threonine. The applicants are simply at a loss to explain why the examiner believes that claim 6 of U.S. Patent 6,596,516 anticipates the presently claimed invention. Accordingly, the applicants respectfully submit that their claimed invention is patentably distinct, and is neither anticipated, nor obvious over claim 6 of U.S. Patent No. 6,596,516. In view of the



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foregoing amendment and remarks, the applicants submit the rejection of claims 6-8, 10-14, 16-18 under the judicially created doctrine of obviousness type double patenting is moot and should be withdrawn, and a rejection of new claims 19-60 would be improper.

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**IV. CONCLUSION**

In view of the foregoing, the claims are now believed to be in form of allowance, and such action is hereby solicited. If any point remains at issue which the examiner feels may be best resolved through a personal or telephone interview, please contact the undersigned at the telephone number below.

Respectfully submitted,  
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